Amendments to the Specification

Please replace the paragraph beginning at page 3, line 22, with the following redlined paragraph:

Figure 6 shows nuclear translocation of NF-κB in THP-1 cells (monocyte cell line) untreated (from left, first panel, images; second panel, quantitation of first panel images) and treated with LPS (third panel, images; fourth panel, quantitation of third panel images). Images are from darkfield, NF-κB labeled, brightfield, and 7-AAD nuclear ~~label~~ labeled.

Please replace the paragraph beginning at page 4, line 6, with the following redlined paragraph:

Figure 11 shows images of nuclear translocation of NF-κB in adherent A-549 cells untreated (from left, first panel, images; second panel, quantitation of first panel images) and treated with IL-1β/TNF-α (third panel, images; fourth panel, quantitation of third panel images). Images are from darkfield, NF-κB labeled, brightfield, and 7-AAD nuclear ~~label~~ labeled.

Please replace the paragraph beginning at page 14, line 16, with the following redlined paragraph:

By way of background and wishing to be bound by theory, NF-κB resides predominantly in the cytoplasm in resting cells. Activating treatments (*e.g.*, IL-1 β /TNF-α or LPS) induce NF-κB translocation into the nucleus in responsive cell types. Thus, the ratio of nuclear to cytoplasmic ~~NFkB~~ NF-κB increases with LPS treatment. Similar to the A-549 cells, NF-κB is translocated from the cytoplasm to the nucleus when the non-adherent human monocyte cell line, THP-1, is exposed to lipopolysaccharide (LPS). Using the identical probing protocol and CCF, again a quantifiable difference in the nuclear localization NF-κB is demonstrated when comparing untreated and LPS-

treated cells (*see* Figures 6 and 9). A nuclear and NF-κB pixel signal correlation analysis CCF was used to quantitate the difference between untranslocated NF-κB and NF-κB translocated to the cell nucleus. The CCF distinguished location-specific (nuclear and cytoplasmic) quantitation of NF-κB to distinguish LPS-treated from untreated THP-1 cells. Thus, the methods of the present disclosure may also be used with non-adherent cells and cell lines.

Please replace the section beginning at page 17, line 1, with the following redlined section:

A. Materials

01. anti-NFκB (F6) : Santa Cruz Biotechnology (Cat. No.SC-8008), 200 μg/ml

02. Alexa Fluor488 donkey anti-mouse IgG: Molecular Probes ~~(Cat),~~ 1.1 mg/ml

03. Streptavidin Alexa Fluor 488: Molecular Probes

04. Recombinant human TNF-α : BD (Cat# 554618, Lot# 0000056653)

05. Recombinant human IL-1□: ~~ebi0science~~ eBioscience (Cat# 14-8018-62~~, Lot#~~)

06. A549 cells (ATCC No. CCL-185)

07. Dulbecco's MEM

08. Fetal Calf Serum

09. F-25 Culture Flask

10. 0.25 % trypsin / EDTA

11. Phosphate buffered saline without Ca^{2+}/Mg^{2+} (PBS)

12. 4% PFA/PBS (Fixation Buffer)

13. 0.1% triton X-100/PBS (Perm Buffer)

B. Cell preparation

We used A549 cells cultured in Dulbecco's MEM supplemented with 10% fetal calf serum in an incubator containing 5% CO_2 at 37. A-549 cells were stimulated with or without TNF-α and IL-1β for 45 min to induce nuclear translocation of NF-κB.

01. Culture A549 cells in the T-75 cm^2 culture flask containing 20 ml of the 10% FCS/ Dulbecco's MEM.

02. Stimulate the exponentially growing cells with TNF-α (2.0 ng/ml) and IL-1β (10 pg/ml) for 45 min at 37°C under 5% CO_2 humidified atmosphere.

03. After stimulation, discard media and wash cells with 5-10 ml of PBS.

04. Add 2 ml of 0.25 % trypsin / EDTA to cells, and incubate 37°C for 1 min or until cells have detached.

05. Suspend cells by adding 8 ml of complete DMEM.

06. ~~transfer~~ Transfer the cell suspension to 15 ml centrifuge tube.

07. Centrifuge at 300 x g ~~1o'~~ 10', 4°C, and remove media.

08. Fix cells by resuspending ~~@~~ at 10^7 cells/ml in 4% PFA/PBS 30', 4°C.

09. Wash with PBS, then perm cells by resuspending ~~@~~ at 2 x 10^7 cells/ml in 0.1% triton X-100/0.02% EDTA/PBS (Perm) 30', 4°C.

10. Add equal volume of anti-NF⊟κB 20 μg/mL in Perm (final mAb concentration of 10 μg/mL) 15', 4°C.

11. Wash Perm Buffer.

12. Resuspend 10^7 cells/ml in Perm + AF488 donkey anti-mouse IgG (10 μg/mL) 15', 4°C.

13. Filter 70 μm mesh and wash with Perm.

14. Resuspend 5 x 10^7 cells/ml Perm + 10 μM 7-AAD 5' and run directly on the ImageStream.

EXAMPLE 2

INDUCTION OF TRANSLOCATION IN NON-ADHERENT CELLS

Human monocyte cell line THP-1, obtained from ATCC (Rockville, MD), were maintained in RPMI 1640 (Gibco, Grand Island, NY) containing 5% fetal bovine serum, 1 mM sodium pyruvate (Mediatech, Herndon, VA), 100 μM nonessential amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (BioWhittaker, Walkersville, MD) in 5% CO_2 atmosphere at 37°C. The density of exponentially growing cells was less than $3x10^5$ cells per ml at the time of all treatments. To induce NF-κB translocation into the nucleus from the cytoplasm, cells were treated for 1 hr with LPS.

The following is the experimental procedure for LPS-induced Nuclear Translocation of NF-κB in THP-1 cells.

Samples:

1) Unstained and single fluorescent color control samples – start with 3.0×10^6 total cells each. In this experiment, controls are:

unstained

NFκB Alexa Fluor488

7-AAD

At the end, resuspend in 100 μl 0.1% triton X-100/PBS.

Unstained and NFκB can be mixed and run as one file, then a separate .rif of unlabeled cells can be created in IDEAS. The 7-AAD control must be run separately, because 7-AAD comes off of labeled cells and stains unlabeled cells, confounding compensation. Furthermore, we run the sample with 7-AAD in the buffer to increase staining intensity (washing it away reduces the intensity about four-fold).

2) Experimental samples – start with 10^7 total cells for untreated LPS-treated. Stain according to following protocol.

~~C.~~A. Materials

~~1~~1. anti-NFκB (F6) : Santa Cruz Biotechnology (Cat. No.SC-8008), 200 ~~u~~μg/ml

~~1~~2. Alexa Fluor488 donkey anti-mouse IgG: Molecular Probes ~~(Cat)~~, 1.1 mg/ml

~~1~~3. Streptavidin Alexa Fluor 488: Molecular Probes

~~1~~4. Lipopolysaccharide (LPS) from E. Coli 0111B4 : Sigma (Cat# L2630~~, Lot#)~~

~~1~~5. THP-1 cells

~~1~~6. RPMI

~~2~~7. Fetal Calf Serum

~~2~~8. T-75 cm^2 Culture Flask

~~2~~9. EDTA

~~23~~10. Phosphate buffered saline without Ca^{2+}/Mg^{2+} (PBS)

~~24~~11. 4% PFA/PBS (Fixation Buffer)

~~25~~12. 0.1% triton X-100/PBS (Perm Buffer)

~~D.~~B. Cell preparation

We used THP-1 cells cultured in RPMI supplemented with 10% fetal calf serum in an incubator containing 5% CO_2 at 37. THP-1 cells were stimulated with or without LPS and for 60 min to induce nuclear translocation of NF-κB.

~~1~~5. Culture THP-1 cells in the T-75 cm^2 culture flask containing 50 ml of the 10% FCS/ RPMI ($3x10^5$ cells/mL).

~~1~~6. Stimulate the exponentially growing cells with LPS for 60 min at 37°C under 5% CO_2 humidified atmosphere.

~~1~~7. Centrifuge at 300 x g ~~1~~0'~~-~~10', 4~~°°~~C, and remove media.

~~1~~8. Fix cells by resuspending ~~@~~ at 10^7 cells/ml in 4% PFA/PBS 30', 4~~°°~~C.

~~1~~9~~5~~. Wash with PBS, then perm cells by resuspending ~~@~~ at 2 x 10^7 cells/ml in 0.1% triton X-100/0.02% EDTA/PBS (Perm) 30', 4~~°~~°C.

~~2~~0~~6~~. Add equal volume of anti-NFκB 20 μg/mL in Perm (final mAb concentration of 10 μg/mL) 15', 4~~°~~°C.

~~2~~1~~7~~. Wash Perm Buffer.

~~2~~2~~8~~. Resuspend 10^7 cells/ml in Perm + AF488 donkey anti-mouse IgG (10 μg/mL) 15', 4~~°~~°C.

~~2~~3~~9~~. Filter 70 μm mesh and wash with Perm.

~~24~~10. Resuspend 5 x 10^7 cells/ml Perm + 10 μM 7-AAD 5' and run directly on ImageStream.